

# Production and fate of dimethylsulfide and dimethylsulfoniopropionate in pelagic mesocosms: the role of sedimentation

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**ABSTRACT:** Elevated concentrations of dimethylsulfide (DMS) sometimes occur in sea water during phytoplankton blooms. To determine the factors controlling the concentration of DMS in sea water, the development and fate of a bloom dominated by *Phaeocystis* sp. was studied in relation to the production and fate of DMS and its precursor  $\beta$ -dimethylsulfoniopropionate (DMSP) in a pelagic mesocosm experiment. The part of this study described here focused on the role of sedimentation of algae as a loss factor for DMSP and as a trigger mechanism for DMS production. A hypothesis tested was that high DMS concentrations occur after a mass release of DMSP from algae due to mass sedimentation followed by cell lysis on the mesocosm floor. This was studied by monitoring *Phaeocystis* cell numbers, chlorophyll *a*, particulate DMSP, dissolved DMSP and DMS in the water column, and by daily measurements of the sedimentation of *Phaeocystis* cells. We evaluated a technique for measuring DMSP indirectly as acrylate by high-performance liquid chromatography (HPLC). This method seemed to be hampered by DMSP-lyase activity of *Phaeocystis* sp. during sample processing, but gave good results when *Phaeocystis* was not dominant. Sedimentation rates of *Phaeocystis* sp. were found to be high and constantly related to the standing stock in the water column. The average sinking velocity of *Phaeocystis* cells was  $1.4 \text{ m d}^{-1}$ . At the decline of the bloom, sedimentation accounted for approximately 50% of the observed loss of *Phaeocystis* biomass, which indicates that cell lysis may have been important as well at this time. The decline of the bloom did not result in an elevated DMS concentration. However, a significant peak in DMS was observed at the end of the exponential growth phase of the *Phaeocystis* bloom. It was concluded that the decline of a *Phaeocystis* bloom does not lead to an elevated DMS concentration in the water column. The continuously high sedimentation of living *Phaeocystis* cells suggests that, in these mesocosms, lysis after sedimentation was an important mechanism for release of DMSP from cells and, hence, production of DMS. Nevertheless, this mechanism in itself can not explain the strong fluctuations in the DMS concentration observed in this experiment.

**KEY WORDS:** *Phaeocystis* · Sedimentation · DMS · DMSP

## INTRODUCTION

Dimethylsulfide (DMS) has gained increasing scientific interest due to its proposed role as an 'anti-greenhouse' gas (Charlson et al. 1987, Andreae 1990, Lawrence 1993). Most atmospheric DMS is emitted from oceans and seas (Andreae 1990), where it is formed

mainly from (bio)chemical conversion of  $\beta$ -dimethylsulfoniopropionate (DMSP), a compound that is produced by several marine phytoplankton species (Keller et al. 1989).

No clear relationship exists between phytoplankton biomass and DMS concentrations in sea water (Holligan et al. 1987, Turner et al. 1988, Iverson et al. 1989). This may be caused by fluctuations in the production and fate of both DMSP and DMS. Interspecific variation in DMSP production by algae, fluctuations in the

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intracellular DMSP concentration in algal cells, grazing of algae by zooplankton, demethylation of DMSP to 3-mercaptopropionate, bacterial consumption of DMS, and photochemical oxidation of DMS to dimethylsulfoxide (DMSO) have all been reported to affect marine DMS concentrations (Brimblecombe & Shooter 1986, Dacey & Wakeham 1986, Kiene & Taylor 1988, Keller et al. 1989, Kiene & Bates 1990, Matrai & Keller 1994). In order to gain insight into the complex relationship between phytoplankton biomass and DMS, a pelagic mesocosm experiment was performed, in which the development and fate of an algal bloom was studied in relation to the production and fate of DMS and DMSP.

The study described here focuses on sedimentation, a process that has hardly been studied in relation to DMSP and DMS. Sedimentation of algae and other particles containing DMSP, like faecal pellets from zooplankton, will have 2 effects: it will decrease the concentration of DMSP in sea water, as was indicated by Belviso et al. (1993), and it will supply the benthic system with DMSP, leading to benthic DMS production (Nedwell et al. 1994). Hence, elevated DMS concentrations may occur after mass sedimentation of algae. Mass sedimentation at the end of a bloom has been described for diatoms (Riebesell 1989, Olesen 1993). Kwint et al. (1993) observed peaks in DMS formation after the decline of a diatom bloom in a mesocosm experiment.

Diatoms, however, are not regarded as important DMSP producers (Keller et al. 1989). Hence, our experiments focused on the Prymnesiophyte *Phaeocystis* sp., which is an important DMSP producer (Keller et al. 1989), with a world-wide distribution (Baumann et al. 1994). Elevated concentrations of DMS were reported during blooms of *Phaeocystis* sp. (Barnard et al. 1984, Holligan et al. 1987, Gibson et al. 1990, Liss et al. 1994), but the relation between *Phaeocystis* sp. and DMS formation is still unclear. Liss et al. (1994) suggested that a mass release of DMSP may occur at the decline of a bloom, leading to the observed elevated DMS concentrations.

We tested the hypothesis that these elevated DMS concentrations are caused by a mechanism of sedimentation of *Phaeocystis* sp. at the end of a bloom, followed by a rapid benthic conversion of DMSP into DMS. Van Duyl et al. (1992) and Osinga et al. (1995) found that, in experimental benthic systems, bacteria responded rapidly to addition of fresh algal material dominated by *Phaeocystis* sp. In addition, Stefels & Van Boekel (1993) recently discovered a strong DMSP-lyase like enzyme activity in axenic cultures of *Phaeocystis* sp., leading to DMS production by the algae. These findings support the idea of rapid benthic conversion of DMSP into DMS: if the algae break down on

the bottom, DMSP will be liberated in an environment with both high bacterial numbers and high algal lyase concentrations, resulting in a high DMS production.

There are, however, uncertainties about the fate of *Phaeocystis* cells at the decline of a bloom. Mass sedimentation of *P. pouchetii* was reported by Wassmann et al. (1990). In the German Bight area, Riebesell (1993) also found that sedimentation was the main loss factor for *Phaeocystis* sp. biomass from the upper layer. On the other hand, Van Boekel et al. (1992) found that cell lysis in the water column was the main fate of the *Phaeocystis* sp. spring bloom in the Dutch Coastal Zone. In this paper, we therefore not only estimated the importance of sedimentation in the production and fate of DMS and DMSP, but also studied the role of sedimentation in the development and fate of a *Phaeocystis* bloom.

In another study, carried out simultaneously in these mesocosms, other parameters and processes involved in the biochemical cycling of DMS and DMSP, like zooplankton abundance and bacterial DMS consumption, were monitored. The results of these measurements will be described elsewhere (Quist et al. in press).

## MATERIAL AND METHODS

**Mesocosms.** Four 1380 l plastic bags (polyethylene/polyamide 2-layered foil) were filled with sea water collected on 14 April 1993 in the Marsdiep, The Netherlands (Dutch Coastal Zone, 52° 59' N, 4° 50' E). At the start of the experiment, extra nutrients were added ( $6 \mu\text{mol l}^{-1} \text{PO}_4^{3-}$  and  $40 \mu\text{mol l}^{-1} \text{NO}_3^-$ ) to create the favourable growth conditions for *Phaeocystis* sp. The open bags (Fig. 1) were placed outdoors in a sheltered part of the harbour of Den Helder (located next to the Marsdiep area). The experiment was run between 16 April and 21 May 1993 at field temperature. For a more detailed description of the mesocosms, see Kwint & Kramer (1995).

**Sediment traps and sampling.** In 2 mesocosms, a device was placed with duplicate sediment traps at water depths of 0.5 and 2 m (Fig. 1). The total depth of the mesocosms was 3 m. The traps, perspex cylindrical cores (diameter 7.4 cm, height 20 cm), were emptied daily. In parallel, samples were taken from the water column at depths of 0.5 and 2 m, using 1.0 l plastic bottles which were imbedded in a frame with lead. The frame with the empty bottles was let down into the mesocosm, and the stoppers were removed from the bottles underwater at the desired depth. This sampling started 3 d after the start of the experiment.

Water samples representing the whole depth transect were taken daily as well from these mesocosms, using a peristaltic pump and a sampling tube that

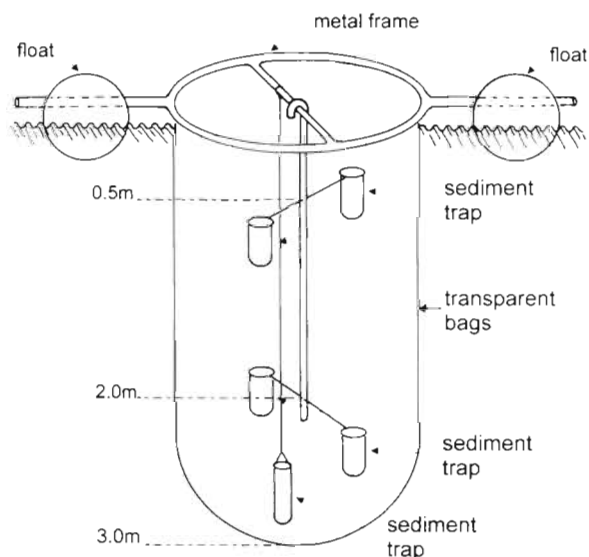


Fig. 1. Mesocosm with sediment traps. Four perspex cylindrical traps were placed in a plastic frame, which could be removed easily from the mesocosms. A further 4 smaller traps (only 1 shown) were fastened with ropes to the mesocosm frame

was randomly moved up and down through the water column.

In both mesocosms, 4 smaller sediment traps (perspex cylindrical cores, diameter 5.4 cm, height ca 35 cm) were placed in the centre of the bags near the mesocosm floor (Fig. 1). These traps were removed 14, 21, 25 and 35 d respectively after the start of the experiment. After removal, the overlying water in the trap was carefully siphoned off, and the volume of the deposited material was determined. On the first day of the experiment, a similar sediment trap was placed outside the mesocosms in the Den Helder harbour at a depth of 3 m. This trap was also removed after 35 d, for comparison of the mesocosm systems with the surrounding water.

All samples were transported immediately to the laboratory for further treatment.

**Analytical procedures.** All samples taken at 0.5 and 2.0 m (water column and sediment traps) were analysed for chlorophyll *a* and *Phaeocystis* cell numbers. Chlorophyll *a* subsamples (subsample volumes ranging between 200 and 500 ml for the water column, and between 30 and 100 ml for the sediment traps, depending on the expected concentration) were collected on glass fibre filters (Whatman GF/F) and measured spectrophotometrically according to Parsons et al. (1984) after extraction with 90% acetone.

For determining *Phaeocystis* cell numbers, 100 ml subsamples were fixed immediately with Lugol solution (1% final concentration) and stored at 2°C. Cell numbers were counted microscopically. Only living

cells were counted. Colourless cells that did not reflect light any more were considered as dead.

The samples taken with the peristaltic pump were used for the determination of the average nutrient concentrations and the average DMS concentration in the water column. Nutrients (nitrate, nitrite, ammonium and phosphate) were measured according to Parsons et al. (1984) on a Technicon auto-analyzer. DMS was measured by gas chromatography (GC) according to Lindqvist (1989), following the preconcentration step described by Kwint & Kramer (1995).

The content of the small sediment traps was analysed for chlorophyll *a*, organic carbon and organic nitrogen. It was very difficult to identify the *Phaeocystis* cells in this material. Hence, accurate determination of the cell numbers in this material was not possible. For the determination of organic carbon and nitrogen, subsamples of 3 to 10 ml were oven-dried for 24 h at 60°C. The dried samples were analysed on a Carlo Erba NA 1500-2 elemental analyser after acidification with 6% sulfurous acid (Verardo et al. 1990).

**High-performance liquid chromatography (HPLC) analysis of acrylate.** Under alkaline conditions (pH 10), chemical cleavage of DMSP occurs, which yields DMS and acrylate in an equimolar ratio (Dacey & Blough 1987). This process is used for indirect measurements of DMSP concentrations: NaOH is added to a sample to induce chemical conversion. Subsequently, the DMS produced by this reaction is measured by GC. We will here evaluate a HPLC technique for measuring DMSP indirectly as acrylate.

Samples for the HPLC analysis were filtered under slight vacuum pressure over glass fibre filters (Whatman GF/F, diameter 47 mm) to separate the dissolved from the particulate fraction. Vacuum pressure never exceeded 10 cm Hg in order to minimize cell disruption. Biological conversion of DMSP to acrylate and DMS may occur in the samples during processing and storage, a process that will lead to an underestimation of the actual DMSP concentration in a sample. Stefels & Van Boekel (1993) showed that the algal lyase activity of *Phaeocystis* sp. could be efficiently inhibited by heat. Hence, the filters were incubated for 15 min at 60°C immediately after filtration to denature the enzymes responsible for the cleavage reaction. After the incubation, the samples were stored at -30°C until further processing.

Two samples of 1.9 ml were taken from the filtrate for measurement of dissolved acrylate and DMSP. These samples were not heated, since most of the enzyme-activity was assumed to be particle-bound: Stefels & Van Boekel (1993) found very little lyase activity in GF/F filtered samples of an axenic *Phaeocystis* sp. culture. One of the 1.9 ml samples was incubated with 100 µl 10 M NaOH (0.5 M final con-

centration) for at least 15 min at room temperature for chemical conversion of DMSP to acrylate and DMS. After the incubation, 100  $\mu$ l 10 M HCl was added to neutralize the pH in the sample. Both samples were stored at  $-30^{\circ}\text{C}$  in plexiglass tubes (Sartedt) until analysis.

The filters were put into a homogenisation flask together with 4.4 ml distilled water and glass beads (diameter 0.45 to 0.5 mm) and were homogenized for 30 s in a  $\text{CO}_2$ -cooled Braun 853038 cell homogeniser. The water content of a wet 47 mm GF/F filter is estimated as 0.6 ml, so that the addition of 4.4 ml distilled water results in a total volume of 5 ml containing all particulate DMSP/acrylate from the original sample. This preconcentration step has to be accounted for when calculating the original concentration in the sample. Distilled water was used instead of filtered sea water to minimize peaks in the chromatogram that are caused by sea salt. These peaks may otherwise interfere with the acrylate peaks.

The homogenized suspension was filtered through a Whatman GF/F glass fibre filter. A 1.9 ml subsample of the filtrate was treated with NaOH as described previously and stored at  $-30^{\circ}\text{C}$  until analysis. The remainder of the filtrate was stored at  $-30^{\circ}\text{C}$  without further treatment.

Analysis of these samples was performed on a Waters 600E HPLC system, using a Waters 994 photodiode array detector, a Rosil C18 5 $\mu$  HL (high loaded) column (150 by 4.7 mm), and 25% (v/v) methanol in distilled water as the carrier fluid. This photo-absorption detection method detects the acrylate as acrylic acid. To prevent dissociation of the acrylic acid, the carrier fluid was acidified with phosphorous acid to a pH of 2. The flow rate of the carrier fluid was  $1.0 \text{ ml min}^{-1}$ .

A sample volume of 100  $\mu$ l was injected into the HPLC with a Hamilton syringe. To prevent pollution of the column, all samples were filtered through a 0.2  $\mu$ m cellulose acetate filter just before injection into the HPLC.

The system was calibrated with standard acrylic acid (Aldrich Chemicals) diluted in 10% 0.2  $\mu$ m filtered aged sea water in distilled water. The aged sea water was added to the standard to create a medium with a similar sea water content as the particle-bound acrylate/DMSP samples, in which 4.4 ml distilled water was added to 0.6 ml sea water. A broad spectrum measurement was performed on 1 of the calibration samples to assess the optimal detection wavelength. All other samples were analyzed at a detection wavelength of 205 nm.

DMSP concentrations were calculated by subtracting the measured acrylic acid concentrations in the untreated samples from the concentrations in the samples treated with NaOH.

The HPLC analysis of acrylate was only performed on samples from 2 m water depth. The volume of the subsamples used for filtration depended on the expected concentration, and were generally equal to the volumes filtered for chlorophyll a analysis.

Particulate DMSP in the water column was also measured by GC as DMS after chemical conversion with NaOH, and these results were used to calibrate the HPLC measurements. The GC-DMSP measurements were performed following the procedures described by Stefels & Van Boekel (1993).

**Calculations.** The amount  $X$  of a compound 'X' collected in the sediment traps after a period  $t$  is a function of the sinking velocity  $v_s$  of 'X', the horizontal surface of the trap entrance  $A$  and the average concentration  $C_{\text{avg}}$  of 'X' in the water column directly over the trap:

$$X(t) = v_s \times t \times C_{\text{avg}} \times A \quad (1)$$

Hence, the sinking velocity of 'X' can be calculated as follows:

$$v_s = X(t)/(t \times C_{\text{avg}} \times A) \quad (2)$$

For each day, the sinking velocity of *Phaeocystis* cells was thus calculated from the results of the microscopic counts.  $C_{\text{avg}}$  was calculated as the average of the cell numbers measured in the water column on 2 successive days.

There was no tidal mixing inside the mesocosms. Due to the sheltered location, there was also little wave-interaction. It was therefore assumed that resuspension of deposited material hardly occurred in these mesocosms. This allows the following simple calculation of sedimentation rates:

$$S(X) = X/A \quad (3)$$

where  $S(X)$  = the sedimentation of compound 'X'  $\text{m}^{-2}$ ;  $X$  = the amount of compound 'X' in the trap;  $A$  = the horizontal surface area of the sediment trap entrance.

For calculation of daily sedimentation rates, a modification of Eq. (3) is used:

$$S_d(X) = [X(i) - X(i-1)] / A \quad (4)$$

where  $S_d(X)$  = the sedimentation of compound 'X'  $\text{m}^{-2} \text{d}^{-1}$ ;  $X(i)$  = the amount of compound 'X' in the trap on Day 'i';  $X(i-1)$  = the amount of compound 'X' in the trap when it was installed on Day 'i-1'. This amount was calculated as the concentration in the water column on Day 'i-1' times the volume of the sediment trap. Missing values for the concentration in the water column were interpolated.

Addition of the values for daily sedimentation shows the total amount of compound 'X' on the mesocosm floor after a period longer than 1 d. However, decomposition of deposited material may occur during the experiment, decreasing the amount of material on the mesocosm floor. The data from the small traps at the

mesocosm floor, which were not emptied daily, gave an indication of the decomposition rate when they were compared to the cumulative daily sedimentation.

When  $S_d(X)$  has been determined, the daily net production ' $P_n$ ' of compound 'X' in the water column can be calculated as follows:

$$P_n = C(i) - C(i-1) + [S_d(X) \times V] \quad (5)$$

where  $C(i)$  = the concentration of compound 'X' in the water column on Day 'i';  $C(i-1)$  = the concentration of compound 'X' in the water column on Day 'i-1';  $V$  = the volume of the water column over  $1 \text{ m}^2$

## RESULTS

In the water column of these mesocosms, no obvious differences were observed between the *Phaeocystis* cell numbers at 0.5 and at 2.0 m. The log of the ratio of the total amount of *Phaeocystis* cells counted over the whole 35 d period at 0.5 m and the total cell numbers counted at 2.0 m did not differ significantly from 0 (paired *t*-test, 2 pairs,  $\alpha = 0.05$ ). Therefore, cell numbers in the water column are presented as the averages of the 2 depths (Fig. 2).

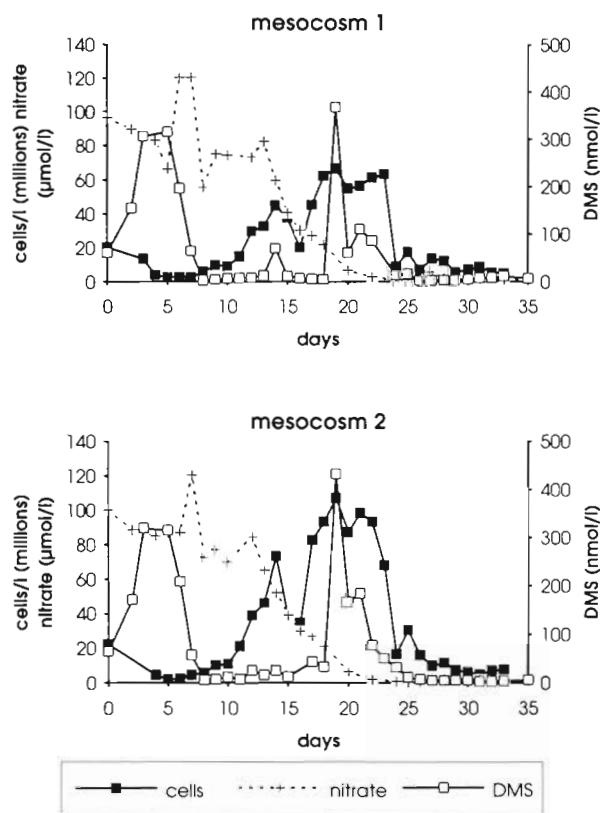


Fig. 2. Development of the *Phaeocystis* bloom and the concentrations of nitrate and DMS in the mesocosms

Although the cell numbers in Mesocosm 1 were in general lower than in Mesocosm 2 (Fig. 2), the patterns are rather similar in both cases. A drop in cell numbers was observed a few days after the experiment started. After 10 d, *Phaeocystis* sp. started blooming, reaching the highest cell numbers between Days 17 and 23, when values up to 70 million cells  $\text{l}^{-1}$  (Mesocosm 1) and 115 million cells  $\text{l}^{-1}$  (Mesocosm 2) were found. The bloom declined at Day 24. Thereafter, cell numbers remained low until the end of the experiment. The cell counts did not distinguish between colonial and non-colonial cells. It was however observed that colonies were abundant throughout the blooming period.

Fig. 2 also shows the concentration of nitrate in the water column. This concentration shows a strong decrease during the blooming period of *Phaeocystis* sp., with the lowest levels occurring at and after the decline of the bloom.

The measured DMS concentrations are shown in Fig. 2 as well. *Phaeocystis* cell numbers showed no correlation with DMS. High DMS concentrations were found between Days 2 and 7, and a peak was observed on Day 19. After Day 19, the DMS concentration remained elevated for a few days before it dropped to background levels. This pattern was strikingly similar in both mesocosms.

The chlorophyll *a* concentration in the water column (data not shown) did not follow exactly the same pattern as *Phaeocystis* sp. In Fig. 3, the ratio between *Phaeocystis* cells numbers and the measured chlorophyll *a* concentrations in the water column is plotted versus time. The ratio was highest in the periods before and after the bloom of *Phaeocystis* sp. When *Phaeocystis* cell numbers were highest, the ratio dropped to values around  $0.2 \text{ pg cell}^{-1}$ . After the bloom, the ratio in Mesocosm 1 was considerably higher than the ratio in Mesocosm 2.

*Phaeocystis* cell numbers in the sediment traps in both mesocosms at 0.5 and 2.0 m are presented in

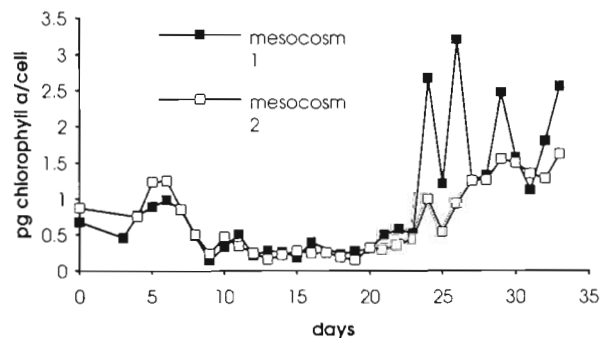


Fig. 3. Ratio between *Phaeocystis* sp. and chlorophyll *a*, expressed as the amount of chlorophyll *a* per cell



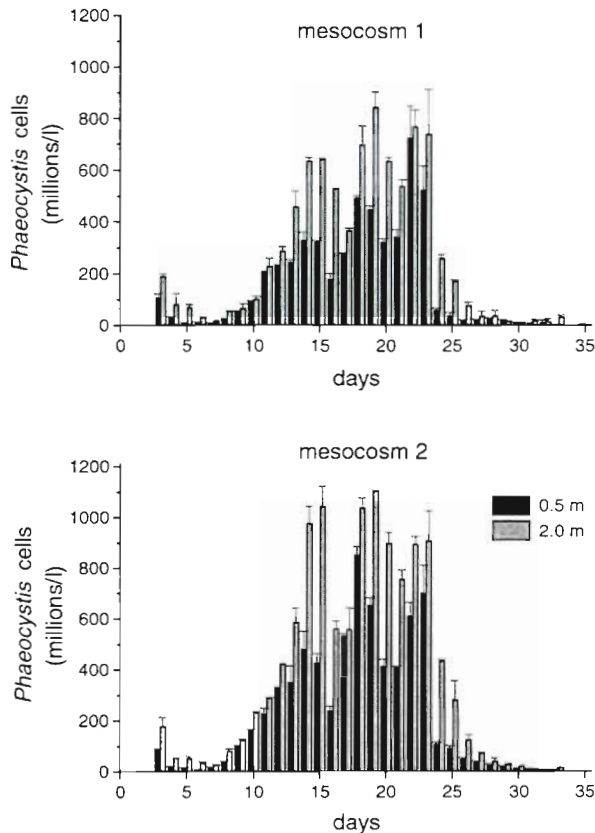


Fig. 4. Numbers of *Phaeocystis* cells measured in the daily removed sediment traps at 0.5 m depth (black columns) and 2.0 m depth (grey columns). Error bars indicate the standard deviation between the duplicate traps

Fig. 4. The pattern in Fig. 4 is rather similar to the development of *Phaeocystis* cells in the water column (Fig. 2), suggesting that sedimentation is directly related to the standing stock in the water column. In contrast to the cell numbers in the water column, a significant difference (paired *t*-test of the log of the ratio of 2.0 and 0.5 m, 2 pairs,  $\alpha = 0.05$ ) was found between the upper and the lower sediment traps: at 2.0 m depth, the total amount of cells collected in the traps over 35 d was 70% higher than at 0.5 m depth. A similar situation was found for chlorophyll *a* (data not shown): although no depth gradient could be observed in the water column, a significant difference (paired *t*-test of the log of the ratio of 2.0 and 0.5 m, 2 pairs,  $\alpha = 0.05$ ) was found in the sediment traps; the total amount of chlorophyll *a* collected over 35 d in the lower traps was also 70% higher than the amount collected in the upper traps.

This discrepancy between the 2 depths was reflected in the calculated sinking velocities for *Phaeocystis* cells (data not shown), which were in general higher at 2.0 m. As an estimate of the average daily sinking

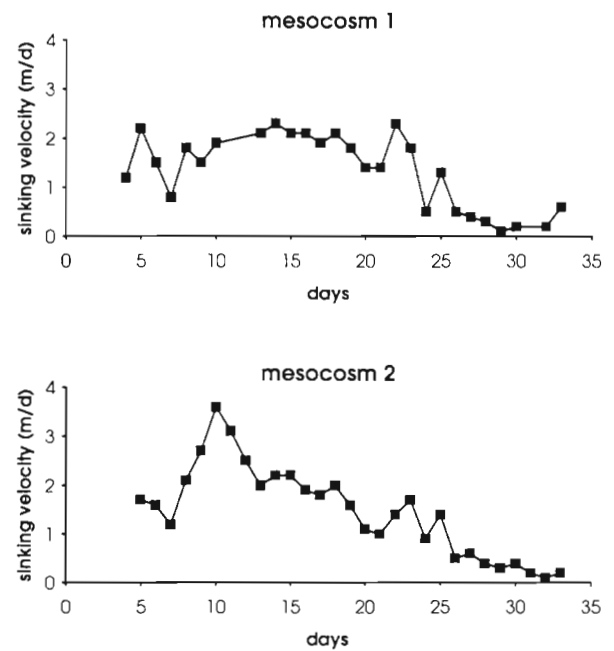


Fig. 5. Average daily sinking velocities ( $\text{m d}^{-1}$ ) of the *Phaeocystis* cells, calculated by averaging the values from 0.5 and 2.0 m

velocities of *Phaeocystis* cells in the mesocosms, the daily averages of 0.5 and 2.0 m were calculated (Fig. 5). These average daily sinking velocities varied between 0.1 and  $3.6 \text{ m d}^{-1}$ . The sinking velocity did not show spectacular fluctuations during the *Phaeocystis* bloom. It did not increase at the decline of the bloom, nor during periods of high DMS formation. The lowest sinking velocities were found at and after the decline of the bloom.

All calculated values together resulted in an average sinking velocity of  $1.4 \text{ m d}^{-1}$  for *Phaeocystis* sp. in these mesocosms. This alone indicates that sedimentation must be quantitatively important in a 3.0 m mesocosm. To show this importance, the accumulation of deposited *Phaeocystis* sp. during the experiment is presented in relation to the development of the cell numbers in the water column (Fig. 6). Because of the differences found between 0.5 and 2.0 m, the sedimentation rates in this figure were based on the results of the lower traps, since these are closer to the mesocosm floor and, hence, are more likely to reflect the situation there. Fig. 6 shows that the number of *Phaeocystis* cells on the mesocosm floor already exceeded the standing stock in the water column a few days after the start of the experiment. During the blooming period of *Phaeocystis* sp., the daily loss of cells due to sedimentation was nearly equal to the average standing stock in this period.

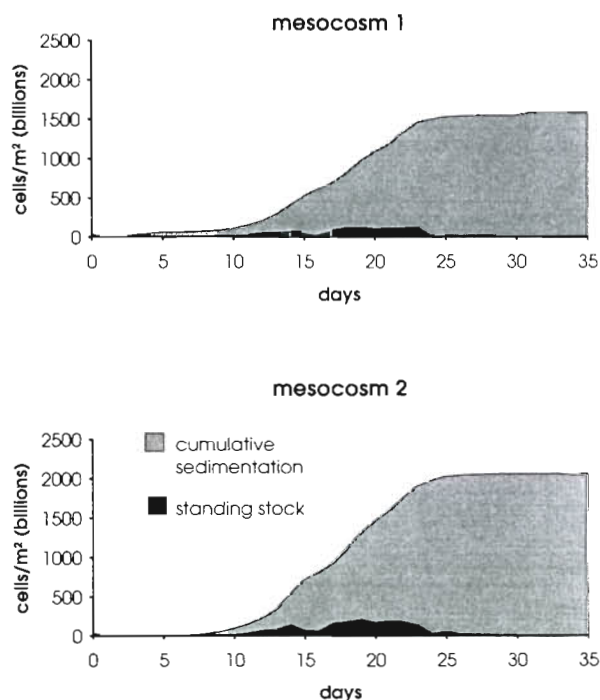


Fig. 6. Comparison of the amount of *Phaeocystis* cells on the mesocosm floor, calculated by addition of daily sedimentation (grey area), with the standing stock in the water column (black area)

Cumulative sedimentation of chlorophyll *a* at 2.0 m depth was compared to the chlorophyll *a* measurements in the smaller traps, expressed per square meter using Eq. (3) (Fig. 7). The measurements (represented by the black bars) were in good agreement with the calculated cumulative values, indicating that the degradation rate of chlorophyll *a* in these traps was low. However, microbial activity did take place in the traps: it was observed that most of the material collected in the traps was black, which indicates sulfide production by sulfate reducing bacteria.

The measurements of organic carbon and nitrogen in the smaller traps were also expressed per square meter. The amount of organic nitrogen on the floor of the mesocosms just after the decline of the *Phaeocystis* bloom (Day 25) was  $5.05 \text{ gN m}^{-2}$  (SD of mesocosms = 0.15). This amount is nearly equal to the measured amount of free inorganic nitrogen present in the water column before the bloom started (Day 7), which was  $5.13 \text{ gN m}^{-2}$  (SD of mesocosms = 0.01).

In Table 1, the results from the measurements at the mesocosm floor at the end of the experiment (Day 35) are compared to the measurements in the sediment trap that had been placed in Den Helder harbour. The sedimentation of chlorophyll *a* in the harbour was comparable to the sedimentation of chlorophyll *a* in the mesocosms. However, the amounts of organic carbon

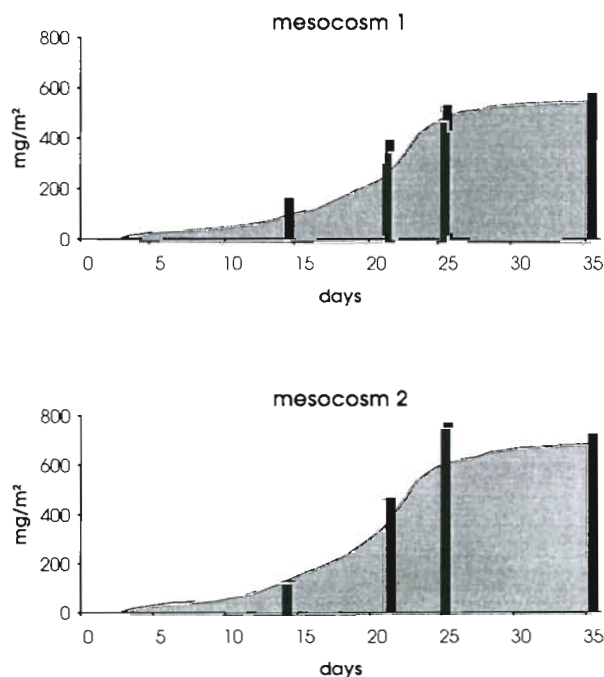


Fig. 7. Accumulation of daily sedimentation of chlorophyll *a* (grey area), compared to the actual amounts of chlorophyll *a* on the mesocosm floor, which were measured in the sediment traps at 3 m depth (black bars)

Table 1. Chlorophyll *a*, organic carbon and organic nitrogen in the trap from Den Helder harbour and in the traps at the mesocosm floor that were removed after 35 d. SD of mesocosms given in parentheses

	Mesocosms	Harbour
Chlorophyll <i>a</i> ( $\text{mg m}^{-2}$ )	655 (75)	670
Organic carbon ( $\text{gC m}^{-2}$ )	28.0 (5.8)	132.1
Organic nitrogen ( $\text{gN m}^{-2}$ )	4.1 (0.7)	19.3

and nitrogen in the harbour-trap were considerably higher, suggesting an additional input of refractory material into this trap.

Net production of *Phaeocystis* sp., estimated from the daily sedimentation using Eq. (5), is presented in Fig. 8. The peak values for DMS (Fig. 3) were not reflected in the pattern for net production. A negative net production was found on Day 24, at the decline of the bloom.

#### HPLC measurements of acrylic acid and DMSP

Under the given conditions, the retention time for acrylic acid in the HPLC was between 2.9 and 3.1 min. The retention time depends on the methanol concen-

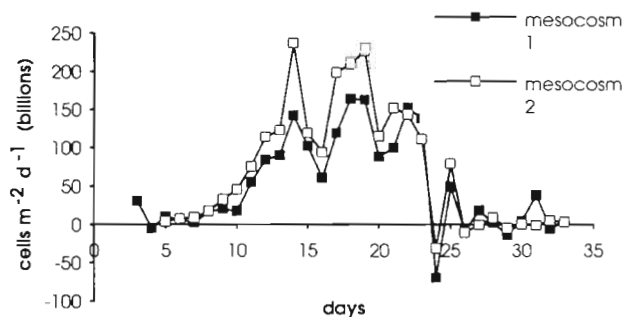


Fig. 8. Calculated rates of daily net production of *Phaeocystis* sp. during the experiment. Negative rates indicate a decrease in *Phaeocystis* biomass that cannot be explained by sedimentation only

tration in the carrier fluid: higher methanol concentrations decrease the retention time. The detection limit for this method is about 1  $\mu\text{M}$ . The concentrations of dissolved acrylate and DMSP were below this detection limit, except for a few samples from the sediment traps.

The results of the HPLC measurements of particle-bound acrylate and DMSP in the water column are shown in Fig. 9, in which the concentrations of both

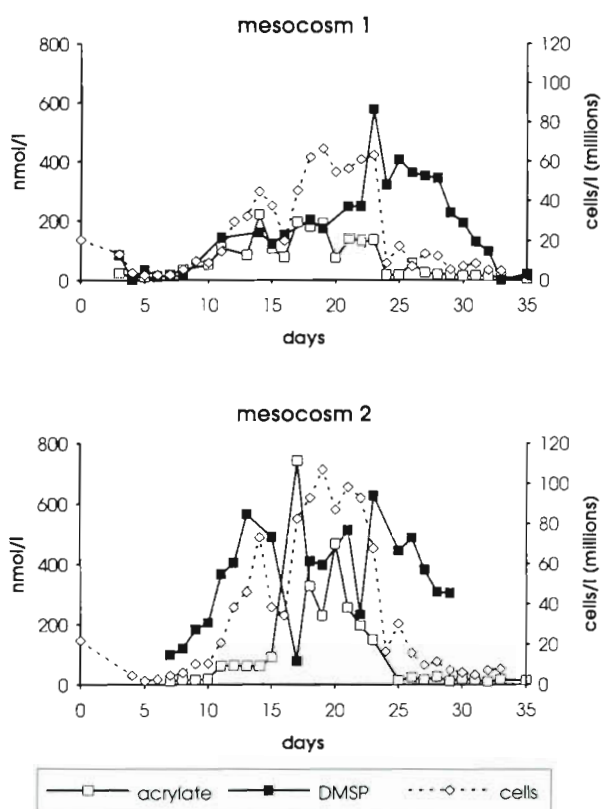


Fig. 9. Concentrations of particle-bound acrylate and DMSP in the water column (at 2.0 m depth) as measured by HPLC analysis. *Phaeocystis* cell numbers are also plotted

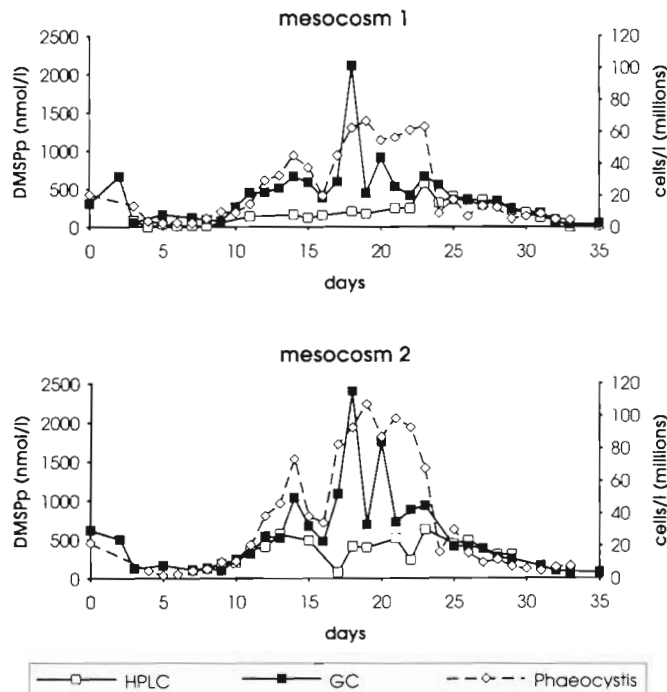


Fig. 10. Concentrations of particulate DMSP in the water column, calculated from both HPLC measurements of acrylate (measured at 2.0 m depth) and GC measurements of DMSP. To show the relation with the occurrence of *Phaeocystis* sp., *Phaeocystis* cell numbers are plotted here as well

compounds are plotted together with the *Phaeocystis* cell numbers in the water column. Although elevated DMSP concentrations were found during the blooming period of *Phaeocystis*, the curves of DMSP and *Phaeocystis* cell numbers show different shapes: DMSP was also found in considerable amounts after the *Phaeocystis* bloom. In contrast, acrylate seemed to be produced mainly in the periods with high *Phaeocystis* cell numbers.

The results from the sediment traps (data not shown) were used to calculate the sinking velocities for DMSP (Eq. 2). This resulted in averages of 1.72  $\text{m d}^{-1}$  and 1.76  $\text{m d}^{-1}$  in Mesocosms 1 and 2 respectively, which is similar to the average sinking velocities for *Phaeocystis* cells calculated from the lower traps (Table 1).

Intercalibration of the HPLC measurements of particle-bound DMSP with the values measured by GC analysis resulted in Fig. 10. Both techniques yielded comparable results in the early phase and in the end phase of the experiment. In between these phases, a large discrepancy between the 2 methods occurred: the values measured by GC were considerably higher in this period, and showed much greater variation. This discrepancy coincided with the occurrence of high *Phaeocystis* cell numbers, which are also plotted in Fig. 10.



The GC measurements of DMSP showed a strong increase of particulate DMSP at Day 18, followed by an equally strong decrease at Day 19. The same phenomena occurred at Days 20 and 21, although the differences in the concentration were not as high.

## DISCUSSION AND CONCLUSIONS

### Methodological considerations

The mesocosms are a useful tool for mimicking a phytoplankton bloom. Under the given experimental conditions, a bloom of *Phaeocystis* sp. was successfully simulated: the observed maximum cell numbers in the mesocosms were comparable to field measurements (Cadée & Hegeman 1986, Van Boekel et al. 1992). The duplicate mesocosms gave comparable results: although the rates and concentrations were in general lower in Mesocosm 1, the observed patterns showed a remarkable similarity, indicating that the development in Mesocosm 1 was in phase with Mesocosm 2.

The method for measuring acrylate in sea water gives good results for concentrations higher than 1  $\mu\text{M}$ . Acrylate concentrations in the field are expected to be much lower: if acrylate is produced from DMSP in a 1:1 ratio with DMS, the *in situ* concentration will be in the same order of magnitude as the DMS concentration, which is generally far below 1  $\mu\text{M}$  (Cooper & Matrai 1989). The technique is therefore not suitable for measuring dissolved acrylate and DMSP, since these have to be detected directly in a sea water sample. Our measurements of the dissolved fraction in the mesocosms confirm this conclusion. The technique can however be useful to measure particle-bound acrylate and DMSP, which are easily preconcentrated on a filter.

Although it is not as sensitive as GC measurements of DMSP via DMS, the HPLC method has the advantage that it is less time-consuming. This makes it a reasonable alternative for GC analysis, for instance, for work with algal cultures in which DMSP concentrations are high. The HPLC measurements were in good agreement with the GC measurements as long as *Phaeocystis* sp. was not dominant. The observed discrepancy with GC measurements in the mid phase of the experiment may have been caused by the DMSP lyases of *Phaeocystis* sp. Disruption of cells during filtration could have brought the intracellular DMSP into contact with these enzymes, leading to a rapid cleavage reaction before the filters were heated. In fact, DMS was often smelled during filtration, which supports this idea of rapid cleavage. This cleavage will lead to an underestimation of the DMSP concentration, and may explain the observed correlation between acrylate concentrations and *Phaeocystis* cell numbers.

Although the results suggest a major role for the lyases of *Phaeocystis* sp., bacterial DMSP-lyase activity has also been demonstrated to be a rapid process (De Souza & Yoch 1995). Therefore, a technique to block the enzyme activity immediately after sampling has to be developed to improve the HPLC method. Hence, our HPLC results have to be interpreted with care.

There was another unexplained feature in our results: the homogenous distribution of chlorophyll *a* and *Phaeocystis* cells in the water column was not reflected in the amounts collected in the sediment traps, which were significantly higher at 2.0 m depth. According to Eq. (1), this can only be caused by an increase of the sinking velocity at greater depth. Aggregation processes can increase the sinking velocity of *Phaeocystis* colonies (Wassmann 1994), but it is unclear which role these processes play in a 3.0 m mesocosm. In addition, the material in the 2.0 m sediment traps was visually not different from the material in the 0.5 m traps.

In a sheltered system, it is likely that the standing stock increases with depth: sinking cells will increase the biomass in lower layers, thus increasing the production potential of these layers. If this is true, the standing stock at both depths has not been determined accurately, since no concentration gradient was found. A likely cause for a disturbance of such a concentration gradient is the sampling procedure: if a concentration gradient existed in the water column, it may have been disturbed by the daily removal of the sediment traps, which took always place shortly before the samples from the water column were taken, or by the water sampler itself. Obviously, this has implications for the calculated sinking velocities at 0.5 m, which will have been underestimated, and at 2.0 m, which will have been overestimated. Hence, the average of these 2 depths seems to be the best estimate of the real average sinking velocities in the mesocosms.

### Phytoplankton dynamics, DMS and DMSP

The amounts of chlorophyll *a* collected after 35 d in sediment traps inside and outside the mesocosms were in the same range, indicating that the mesocosm reasonably reflected the algal production in the Marsdiep area, despite the addition of extra nutrients. The much higher amounts of organic carbon and nitrogen collected in the trap outside the mesocosm can be explained by a continuous input of detritus, which probably resuspends from the sea floor due to tidal currents.

Comparison of the cumulative daily sedimentation with the actual amount of chlorophyll *a* at the mesocosm floor at Day 35 showed that the degradation of

chlorophyll *a* was very low compared to the production. This may be caused by oxygen depletion in the sediment traps: chlorophyll *a* is much more persistent under anoxic conditions than under oxic conditions (Sun et al. 1993). The observed sulfate reduction activity confirms that anoxia occurred inside the traps. When this low degradation of chlorophyll *a* is taken into account, the agreement between cumulative daily sedimentation and the content of the traps at the mesocosm floor shows that the simple sediment trap technique that we used here yields reasonably consistent results.

Since organic carbon and nitrogen were not measured in the water column, it is difficult to estimate mineralization rates for these compounds. It is however clear that there is a considerable flux of organic nitrogen to the mesocosm floor. The mesocosm floor will thus play an important role in the nitrogen cycle in these systems.

To estimate the relative dominance of *Phaeocystis* sp. in these experiments, we compared the calculated amounts of chlorophyll *a* per *Phaeocystis* cell in the mesocosms with values measured in *Phaeocystis* cells isolated from the southern North Sea, which varied between 0.13 and 0.60 pg cell<sup>-1</sup> (Buma et al. 1991, Lancelot et al. 1991). The values measured during the *Phaeocystis* bloom in the mesocosms were close to the bottom end of this range, suggesting that the phytoplankton population in this period was almost a monoculture of *Phaeocystis*. The high chlorophyll *a* contents measured before and after the *Phaeocystis* bloom indicate the presence of other species, which was confirmed by microscopic observations of Quist et al. (in press), who found that the diatom *Nitzschia closterium* in particular was present in significant amounts during these periods.

The importance of sedimentation for *Phaeocystis* in this experiment is evident, but this is mainly due to the experimental circumstances. The average sinking velocity of 1.4 m d<sup>-1</sup> indicates that daily losses due to sedimentation in the southern North Sea will be much smaller than in these mesocosms, since the southern North Sea is much deeper and much more turbulent. The value of 1.4 m d<sup>-1</sup> is low compared to the value of 6 m d<sup>-1</sup> that was reported for *Phaeocystis* colonies in the Marsdiep area (Van Boekel et al. 1992). This may be explained by the continuous presence of many non-colonial cells, which have a much lower sinking velocity.

According to Riegman et al. (1992), blooms of colonial *Phaeocystis* sp. mainly occur in areas characterized by a high nitrate availability. The low nitrate concentration in the mesocosms at Day 24 therefore indicates that the *Phaeocystis* bloom in the mesocosms may have declined due to nitrate-limitation. This nutri-

ent stress did not lead to an increased sinking velocity of *Phaeocystis* sp. Only 50% of the observed loss of biomass during the decline of the bloom could be attributed to sedimentation, which explains the negative net production at Day 24. Mass cell lysis will probably have occurred in the water column on this day. This suggests that lysis in the water column due to nutrient stress is the main process terminating the *Phaeocystis* bloom, which is in agreement with the conclusions of Van Boekel et al. (1992).

Since sedimentation was not measured on the first 2 d, it is not possible to say which process caused the observed decline of the phytoplankton population that was present in the Marsdiep water with which the mesocosms were filled. However, before the mesocosms were filled, this water had been stored for 2 d in a dark container. This may have had a negative effect on the algae.

If we assume that the underestimation of the DMSP concentrations measured by HPLC due to enzyme activity is the same in samples from the water column and the sediment traps, the calculated average sinking velocity for DMSP will be reasonably accurate. Since this velocity was similar to the average sinking velocity of the *Phaeocystis* cells, it may be assumed that freshly deposited *Phaeocystis* cells still contained most of their intracellular DMSP. The daily loss of *Phaeocystis* cells due to sedimentation was considerable and constantly related to standing stock in the water column. Hence, the daily flux of DMSP to the mesocosm floor must have been continuously high during the blooming period of this species. As a consequence, release of DMSP and formation of DMS is likely to have occurred to a large extent on the mesocosm floor.

It should be noted here again that in the southern North Sea, sedimentation will have less impact than in the mesocosms. Therefore, it cannot be concluded that the mechanism of DMS production after sedimentation is important in the southern North Sea. Nevertheless, deposition of DMSP due to sedimentation of intact cells may be significant in areas characterised by low turbulence and shallow depths, e.g. in certain areas in the Dutch Wadden Sea. Deposition of DMSP-containing algae seems to occur in stratified areas as well. Nedwell et al. (1994) reported on very high DMSP and DMS concentrations in and above sediments in the northern North Sea.

In the mesocosms, the continuously high sedimentation of *Phaeocystis* sp. and DMSP did not lead to continuously high DMS concentrations in the water column. This points to other factors involved in the regulation of the DMS concentration. Microbial activity may be important in this regard. Kiene & Bates (1990) reported a high bacterial turnover rate for DMS. In addition, Kiene & Service (1991) found that less than 30% of the

DMSP produced in estuarine waters was converted to DMS. Hence, the DMS concentration in sea water will usually be much lower than the concentration of particulate DMSP. However, when this close coupling between DMSP production and microbial consumption of DMSP and DMS is disturbed for whatever reason, accumulation of DMS may occur. Such an accumulation took place during the first days of our experiment, when the concentration DMS was rather high relative to the amount of particulate DMSP. The decline of the phytoplankton population present in the initial water may have caused a sudden increase in the concentration of dissolved DMSP followed by rapid cleavage of DMSP by the DMSP lyases, which may still have been present in the decaying algae. The growth of the DMS(P) consuming bacteria may not have equalled this rapid production of dissolved DMSP and DMS. Low microbial consumption rates of DMSP and DMS may thus have enabled these relatively high concentrations of DMS. More details on the role of bacteria in this experiment will be presented by Quist et al. (in press).

Regarding the dominance of *Phaeocystis* sp. around Day 19, the second DMS peak must have originated from DMSP production by this species. This peak did not occur at the decline of the *Phaeocystis* bloom, as was predicted by Liss et al. (1994), but during the late exponential growth phase of the bloom. The mass lysis that probably occurred at the decline of the bloom did apparently not lead to elevated DMS concentrations.

The hypothesis that the DMS peak was caused by a mass sedimentation event can be rejected, since the peak was not preceded by an increased sinking rate of *Phaeocystis* sp. It was therefore concluded that enhanced production of DMSP by the algae must have caused the DMS peak. *Phaeocystis* may increase its DMSP production under nitrogen limitation: DMSP can be used for osmoregulation (Dickson & Kirst 1987), and under nitrogen limitation, production of DMSP may replace the production of other osmolytes that contain nitrogen (Turner et al. 1988). The steep peak in the concentration of particulate DMSP observed on Day 18 indeed suggests elevated DMSP production, which was however followed by a strong release of DMSP on Day 19. The latter does not support the osmolyte theory, since no sudden changes in salinity occurred in this phase of the experiment (data not shown).

Since DMSP-lyase activity was assumed to be responsible for the production of acrylate in the HPLC samples, the amount of acrylate measured may give a rough indication of the activity of these enzymes. The highest values were observed during the exponential growth phase of *Phaeocystis* sp., implying that the capacity to split DMSP is already maximal before the

enhanced production of DMSP takes place. A possible function of this early enzyme activity is that the production of acrylate from DMSP may inhibit grazing by zooplankton. Estep et al. (1990) compared consumption of acrylate by zooplankton with eating hot plastic. These authors reported the observation that the copepod *Calanus finmarchicus* rejected colonies of *Phaeocystis pouchetii* immediately after ingestion. This may have been caused either by high concentrations of acrylate inside the colonies or by release of DMSP by the cells during grazing, followed by a rapid enzymatic production of acrylate.

However, the anti-grazing properties of acrylate can not explain the mass release of DMSP at Day 19, although a possible relation with zooplankton grazing is suggested by Quist et al. (in press). Hence, for a better understanding of the relation between *Phaeocystis* sp. and DMS, future research should focus on the possible functions of DMSP production by *Phaeocystis* sp., and on the specific environmental conditions enhancing this production.

**Acknowledgements.** The authors thank Rikus Kloosterhuis for his assistance with CHN analysis. Fruitful discussions with Herman Ridderinkhof and Geert-Jan Brummer led to a better interpretation of the results from the sediment traps. This study was funded by the Dutch National Research Programm (project NOLK/026/90). NIOZ publication number 3017

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